Point of view

Is the heart period a linear gauge of autonomic neural activity?

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Introduction

The role of abnormalities of autonomic neural activity in the genesis and progression of cardiac disease has been recognized since the early '60s and continues to receive considerable attention. A large body of evidence has extended this role from the facilitation of acute events, such as myocardial ischemia and arrhythmias, to the induction of slowly developing processes which, albeit initially adaptive, may finally contribute to heart failure. Part of this evidence derives from experimental studies in which neural activity or the humoral indexes reflecting it have been directly measured. Nonetheless, a pivotal contribution has been provided by clinical studies. Indeed, in these studies direct measurements were replaced by the evaluation of "autonomic indexes", such as heart rate variability[1] and baroreflex sensitivity[2,3], all derived from the measurement of sinus node cycle length. The use of the sinus cycle length as a surrogate measurement of autonomic neural activity implies the following assumptions: 1) the cycle length is under the control of autonomic nerves; 2) the relation between autonomic activity and cycle length, i.e. the input/output relation of the transduction taking place in the sinus node, is linear. While the former assumption is based on strong evidence, the latter has recently been questioned on the basis of experimental results and critical reconsideration of previous studies. The purpose of this review was to summarize such findings and to discuss their potential impact on the interpretation of commonly used autonomic indexes.

The input/output relation of pacemaker cells

The relation between the cycle length and the neuromediator concentration at the effector synapse may play a central role in establishing how neural discharge translates into the sinus cycle length. This relation has recently been investigated by systematic analysis of the effect of acetylcholine on the pacemaker activity of isolated sinoatrial myocytes[4], which is endowed with spontaneous variability[5]. The pacemaker cycle length was measured, on a beat-to-beat basis, along with all the action potential parameters that concur to it. Time series of values were generated from each parameter and analyzed with the methods peculiar to time-domain analysis of heart rate variability. The results of this study can be summarized as follows:

- acetylcholine prolonged the mean cycle length in a dose-dependent fashion, the relation between the cycle length and acetylcholine concentration strongly diverging from linearity;
- acetylcholine-induced cycle length prolongation was associated with a decrease in the diastolic depolarization rate (DDR), with negligible changes in the threshold potential (V_{th}). The relation between acetylcholine concentration and DDR was linear;
- acetylcholine dose-dependently increased the variability of the cycle length, expressed either as standard deviation (SDNN) or as coefficient of variation (CVNN), but did not modify the variability of DDR and V_{th};
- an increase in the variability of the action potential duration (APD) was also observed, probably as the result of enhanced cycle length variability.

The non-linearity of the relation between cycle length and acetylcholine concentration is implicit in the mechanism by which the former is generated and modulated by acetylcholine. The pacemaker activity of sinoatrial cells depends on the progressive membrane depolarization developing during the diastolic interval (diastolic depolarization) till a voltage threshold necessary in order to elicit the action potential is achieved (Fig. 1A)[4]. The duration of the diastolic interval could be modulated by changing either the DDR (in mV/s) and/or the V_{th}. Consistently with a number of previous observations on the neurohumoral modulation of pacemaking in different preparations[6-9], acetylcholine-induced prolongation of the cycle length mainly resulted from a reduction in the DDR. The cycle length can be related to action potential parameters by the following function:

$$\text{cycle length} = \text{APD} + V_{th}/\text{DDR} \qquad (1)$$

This equation predicts that the cycle length may depend on the V_{th} in a linear fashion, but its relation with the DDR may be hyperbolic. A hyperbolic relation was indeed found experimentally (Fig. 1B) and it implies that the magnitude of the cycle length changes resulting from a given change in the DDR may depend on the initial value of the latter. Thus, DDR fluctuations of equal magnitude will have a larger impact on the cycle length whenever they occur during bradycardia (low baseline DDR) than when they occur during tachycardia (high baseline DDR). This implies that the SDNN may be intrinsically related to the mean cycle length.

The observation that the CVNN, which represents normalization of the SDNN to the mean cycle length, was still increased by acetylcholine is more difficult to explain. In order to clarify this point and to provide a quantitative test for the hypothesis that acetylcholine-induced changes in cycle length variability might be accounted for by the relation described in equation 1, we developed a numerical model[4]. In the latter, cycle length variability was simulated by random fluctuations of the DDR around a mean value, which was subjected to modulation by acetylcholine; moreover, the APD was related to the preceding diastolic interval according to a linear function derived by the interpolation of experimental data. The model was able to accurately reproduce all the experimental findings and provided independent estimates of action potential parameters closely matching the measured values[4]. This led to the conclusion that, due to the shape of the relation between cycle length and DDR, any condition leading to depression of the mean DDR will simultaneously increase the mean cycle length and its variability, expressed either as SDNN or CVNN. Support to this view and further validation of the model were provided by the evidence that other interventions affecting the DDR of sinoatrial myocytes, such as β-adrenergic stimulation and temperature changes, also modulated cycle length variability, in agreement with numerical prediction[4].

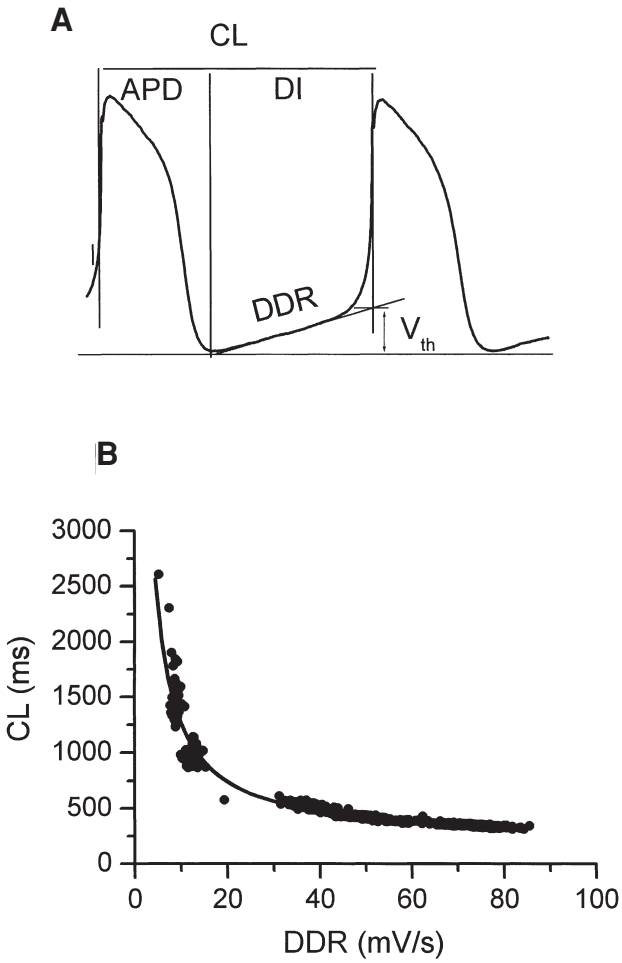

Figure 1. *Relationship between action potential parameters and the sinus node pacemaker cycle.* A: *pacemaker cycle recorded from a spontaneously active sinoatrial myocyte and the parameters determining it.* B: *relationship between the cycle length (CL) and the diastolic depolarization rate (DDR) recorded from a single sinoatrial myocyte during exposure to increasing acetylcholine concentrations* (dots); *the* solid lines *represent the function described by equation 1 (see text). APD = action potential duration; DI = diastolic interval; V_{th} = threshold potential. From Rocchetti et al.[4], with permission.*

The model helped to clarify the mechanism by which also the CVNN may be intrinsically dependent on heart rate. This mechanism can be understood by splitting the cycle length into its two components, i.e. the APD and the diastolic interval, as shown in figure 2. Panel A of this figure shows that 1) the coefficient of variation of the diastolic interval is large, but independent of the cycle length; 2) the coefficient of variation of the APD is small, but increases at longer cycle lengths. While the contribution of the latter to the cycle length-dependent increase in the CVNN may be small, an additional mechanism is provided by the fact that the proportion of the cycle length accounted for by the diastolic interval (having the largest variability) is increased at longer cycle lengths (Fig. 2B). In other words, the CVNN is expected to increase at longer cy-

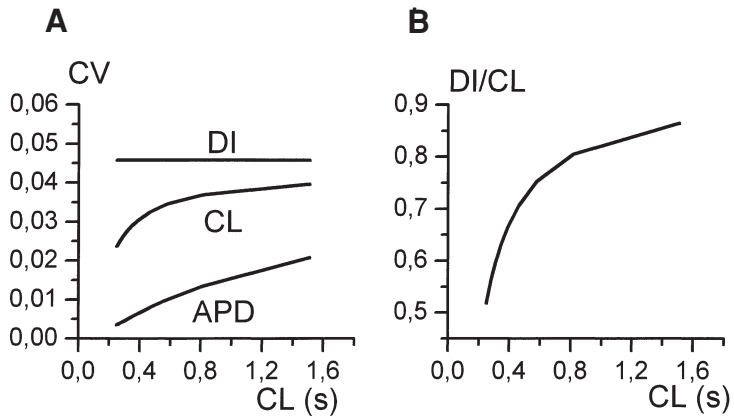

Figure 2. *Model interpretation of the rate-dependency of the coefficient of variation (CV) of cycle length. Simulations were performed by using action potential parameters measured from rabbit sinoatrial myocytes[4].* A: *cycle length dependency of the CV of the diastolic interval, cycle length and action potential duration.* B: *cycle length dependency of the proportion of the pacemaker cycle accounted for by the diastolic interval (see text for explanation). Abbreviations as in figure 1.*

cle lengths, mainly because of an increase in the weight of the cycle component having the largest variability (i.e. the diastolic interval).

To summarize, experimental results and model simulations concur in suggesting that cycle length variability might have an intrinsic dependency on the mean heart rate. In this context the term "intrinsic" signifies that heart rate changes *per se*, whether or not reflecting changes in autonomic activity, may affect heart rate variability. This is a novel interpretation; indeed, a correlation between cycle length variability and the mean heart rate had long been observed, but it was consistently explained on the basis that both indexes were dependent on autonomic activity[10].

Experiments in sinoatrial myocytes showed that, as expected from their effect on pacemaking, muscarinic and β-adrenergic agonists exerted opposing effects on cycle length variability. However, in the clinical setting, cholinergic modulation may be superimposed on a variable degree of sympathetic activation, a condition not tested in our experiments. Thus, the question may arise on how concomitant adrenergic stimulation may affect acetylcholine-induced changes in cycle length variability. Fortunately, the mechanism of interaction between muscarinic and adrenergic receptors in the modulation of the ionic currents underlying the pacemaker function has been clarified to the extent that predictions on this matter are reasonable[11,12]. β-adrenergic and M2-muscarinic receptors compete in regulating the intracellular cAMP concentration, which is also a common step in the modulation of the currents underlying the DDR (mainly I_f) and the V_{th} (mainly I_{CaL})[13]. For this purpose, the role of other cholinergic or adrenergic receptors can be considered negligible. Thus, the effect of muscarinic/adrenergic antagonism on the sinus node can be accurately reproduced by reciprocal changes in DDR and V_{th}, both contributing to change the cycle length. The numerical model predicts that the relation between SDNN and heart rate would be the same whether the changes in heart rate were caused by DDR modulation alone, or by concomitant DDR and V_{th} changes such as those resulting from cholinergic/adrenergic antagonism. This is simply a consequence of the mathematical relation linking DDR and V_{th} to the cycle length and predicts that the rate-dependency of heart rate variability indexes, observed during exposure to acetylcholine alone, should be also present when adrenergic activation is superimposed.

The input/output relation of the sinus node

As mentioned above, the use of the cycle length as a surrogate measurement of autonomic neural activity relies on the postulate that these variables may be linearly related to each other. The ground for such an assumption was provided by studies in which vagal neural activity and heart period were simultaneously measured in anesthetized animals[14,15]. The results of such studies were indeed compatible with a linear relation between the vagal firing rate and the heart period.

How can these findings be reconciled with the hyperbolic dependency of the cycle length on the agonist concentration found in isolated myocytes?

It is theoretically possible that the non-linearity in acetylcholine release as a function of the neural firing rate may exactly compensate the non-linearity of the cell response to the agonist, thus resulting in an overall linear response. Besides being unlikely, this hypothesis cannot be tested because the acetylcholine concentration available at the effector synapse cannot be measured directly and its indirect estimates are largely speculative. A second possibility is that, within the range of the cycle length modulation tested experimentally, the non-linearity of the process might have been overlooked. We considered this possibility by testing whether the (non-linear) numerical model developed from our single cell experiments[4] might re-

produce the observations made in animal studies[16]. The model was applied, with its original parameters, to three classical studies most often quoted to support the linearity of the cycle length as an index of neural activity. All the experimental findings could be satisfactorily reproduced by the non-linear model. In particular, the results of the analysis showed that within a reflex response (e.g. baroreceptor activation) the non-linearity can be hardly detected, but its impact becomes relevant when the same reflex is elicited starting from a different cycle length, a condition that, to the best of our knowledge, has not been tested experimentally.

Altogether, this leads to the conclusion that there is no evidence against the possibility that the non-linearity of the response, detected in single pacemaker elements, might also characterize cycle length modulation by neural activity *in vivo*.

Implications

The non-linearity in the cycle length response to neural activity would translate into an intrinsic rate-dependency of the cycle length variability indexes[4] commonly used in the assessment of autonomic function. On the other hand, changes in the autonomic balance are expected to modulate the heart rate and its variability independently of each other. Thus, the possibility of an intrinsic rate-dependency may generate confusion in the interpretation of autonomic indexes and should be considered with care. This point can be illustrated by the following examples.

In a study by Tsuji et al.[17] on a large cohort of patients from the Framingham study, heart rate was found to be an independent determinant of cycle length variability, accounting for 22.6% of its global variance. The natural log (ln) of SDNN was linearly related to the mean heart rate, with a regression coefficient of -0.17 ms per 10 b/min. Mechanistic interpretations of such a correlation may range between two extremes: 1) the same factor (neural activity) controls both heart rate and SDNN independently; 2) SDNN is an intrinsic function of heart rate, irrespective of the mechanisms (neural or non neural) determining the latter. Although we cannot provide conclusive evidence to discriminate between these hypotheses, it is possible to test whether the rate-dependency of the SDNN might account for the observed correlation in both qualitative and quantitative terms. To this end, we performed model simulations entirely based on parameters measured from sinoatrial myocytes. Such simulations closely reproduced the curvilinear relation between SDNN and heart rate observed in the Framingham population; after ln transformation of the SDNN, the estimated relation was fitted by linear regression with a coefficient -0.16 ms per 10 b/min. This would suggest that the correlation between cycle length variability and the mean heart rate observed in a large population of human subjects may be almost entirely accounted for by the intrinsic rate-dependency of SDNN. Since there is little doubt that autonomic balance may affect the SDNN independently of heart rate, the close similarity between the measured and estimated regression coefficients might seem surprising. Nonetheless, it should be considered that, in the Framingham population, the relation between SDNN and heart rate emerged after the effect of other sources of variance potentially related to the autonomic balance had been removed (by a multifactorial design). This would tend to minimize the rate-independent contribution of autonomic balance to the variance of the SDNN. Thus, although the SDNN may also depend on the autonomic balance, it may be that the regression coefficient reported by Tsuji et al. reflects the intrinsic rate-dependency of cycle length variability. In the light of this, the similarity between the measured and estimated coefficients suggests that the model might provide reasonable quantitative estimates of the rate-dependency of the SDNN in humans. Since the model parameters were measured from myocytes of rabbit, a species with heart rates much higher than those of humans, this may seem surprising. Information from comparative studies of species with a wide range of heart rates suggests that their sinus node action potentials may differ in terms of duration, with the remaining parameters being remarkably constant[18]. A longer action potential may partially account for the longer pacemaking cycle length. To test whether this difference might affect the model predictions on the rate-dependency of the SDNN, we adjusted the action potential parameters in the model so as to simulate those found in the pig, an animal with a heart size and pacemaker rates similar to those of humans. Under these conditions, the relation between the ln of the SDNN and heart rate had a regression coefficient of -0.19 ms per 10 b/min, i.e. still close the one reported for humans by Tsuji et al.

A recent study[19] evaluated the modulation of autonomic indexes by zatebradine, a pure "bradycardic agent", the action of which is based on the selective blockade of the pacemaker current I_f in the sinus node[20]. The drug was found to prolong the cycle length, increase the CVNN and enhance baroreflex sensitivity. However, the ratio between the low and high frequency (LF/HF) spectral components of cycle length variability was not modified by zatebradine. In keeping with the current interpretation of autonomic indexes, it was suggested that changes in the CVNN and in baroreflex sensitivity reflect an effect of zatebradine on autonomic balance[19]. Although possibly at odds with this interpretation, the lack of changes in the LF/HF was not discussed. However, since zatebradine decreases heart rate via its direct effects on the sinus node, the alternative hypothesis should be considered that the drug-induced increase in the CVNN might simply reflect the intrinsic rate-dependency of cycle length variability. The same

argument applies to the baroreflex sensitivity, which is also based on cycle length measurements and may suffer from the same ambiguities discussed for the time-domain heart rate variability indexes[16]. This interpretation would also be consistent with the lack of any drug effect on the LF/HF, an index which we found to be unaffected by the non-linearity of the sinus node input/output relation[16]. Of course, a genuine effect of zatebradine on the autonomic balance cannot be ruled out, but a positive conclusion based on the CVNN and baroreflex sensitivity measurements seems equally unwarranted.

Conclusions

The above discussion leads to the following conclusions: 1) assuming the cycle length to be a linear index of neural activity may be unjustified; 2) if the relation between neural activity and the cycle length were dominated by the non-linear relation observed in isolated sinoatrial myocytes, the time-domain heart rate variability indexes would be intrinsically dependent on the mean heart rate. It is fair to stress that, although several observations are consistent with it, the latter hypothesis remains to be proven; thus, the implications of our analysis should be considered as hypothetical. Nonetheless, the analysis highlights the potential consequences of the current assumptions, which are equally unproven and in the light of the observations on single sinoatrial myocytes, likely to be incorrect.

The rate-dependency of the time-domain heart rate variability indexes would be particularly relevant for conditions/interventions potentially associated with changes in the intrinsic pacemaker properties of the sinus node. In such cases the determination of indexes devoid of potential rate-dependency, such as the LF/HF ratio, may help to resolve the ambiguity. On the other hand, it is important to recognize that the concept of the intrinsic rate-dependency of heart rate variability would not be in contrast with the well established notion that heart rate variability may be sensitive to neural autonomic activity and can be affected by it independently of changes in the mean heart rate.

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